

AMENDMENTS

Amendments to the Specification:

I. Please replace the title of the application, located on page 1, line 1, with the following substitute title:

METHOD FOR MODULATING CELL PROLIFERATION IN  
THE SEED COAT AND/OR INTEGUMENT Seeds

II. Please replace the paragraph located on page 21, lines 1–2, with the following substitute paragraph:

**Figure 6** is an alignment of wild-type MNT (SEQ ID NO: 55) and mutant mnt-I cDNAs (SEQ ID NO: 5) from translational start to stop;

III. Please replace the paragraph located on page 21, line 3, with the following substitute paragraph:

**Figure 7** is an alignment of wild-type MNT (SEQ ID NO: 3) and mutant mnt-1 (SEQ ID NO: 6) predicted proteins;

IV. Please replace the paragraph located on page 21, lines 4–5, with the following substitute paragraph:

**Figure 8** is an alignment of *Arabidopsis thaliana* MNT cDNA (SEQ ID NO: 55) with its orthologue in *Brassica napus*, BnARF2 (SEQ ID NO: 9);

V. Please replace the paragraph located on page 21, lines 6-8, with the following substitute paragraph:

**Figure 9** is an alignment of *Arabidopsis thaliana* MNT predicted protein (SEQ ID NO: 3) with its orthologues in *Brassica napus* (oilseed rape) (BnARF2) (SEQ ID NO: 10) and *Oryza sativa* (rice) (OsARF2) (SEQ ID NO: 61);

VI. Please replace the paragraph located on page 23, lines 19-25, with the following replacement paragraph:

The following vectors are used in the examples:

pGEMT (Promega, Southampton, UK)

BJ36, BJ40, BJ60 (gift of Bart Janssen, Horticultural & Food Research Institute of New Zealand)

pART7 (Gleave, 1992)

pFGC5941 (Cambia, Canberra, Australia; ChromDB, <http://www.chromdb.org/plasmids>)

VII. Please replace the paragraph located on page 33, lines 1-24, with the following replacement paragraph:

We mapped the MNT locus to a 60.9 kb region of chromosome 5 that was annotated by The Arabidopsis Information Resource (TAIR) (<http://www.arabidopsis.org>) to contain 17 genes. T-DNA insertion lines with insertions in these genes generated by The Salk Institute Genome Analysis Laboratory (SIGnAL (Alonso et al., 2003) (<http://signal.salk.edu>)) were obtained from the Nottingham Arabidopsis Stock Centre (NASC) (<http://nasc.nott.ac.uk>). Salk line no. 108995 (NASC stock no. N608995), with an insertion in the coding region of the AUXIN RESPONSE FACTOR 2 (ARF2) gene, included a plant homozygous for the insertion with a similar phenotype to mnt-1 mutants, including closed flowers and large seeds (FIG. 5A-C). Genotypic scoring of segregants from the Salk 108995 family, including one heterozygote and the homozygote, is shown in FIG. 5D. Specifically in FIG. 5D Top: Scoring for presence of an

insertion in the ARF2 gene. Primers used were 5' TGG TTC ACG TAG TGG GCC ATC G 3' (SEQ ID NO: 62), and 5' GAG TGG GTG GAG TGT GTT TG 3' (SEQ ID NO: 63). Lanes M and O show presence of the insertion. Bottom: Scoring for homozygous insertion mutants. Primers used were 5' GAG TGG GTG GAG TGT GTT TG 3' (SEQ ID NO: 63) and 5' AGT TGG TTT TCG TTT GAG CAT 3' (SEQ ID NO: 64). PCR conditions are set so that the gene will only amplify if there is no insertion: therefore PCR products will be amplified from DNA extracted from wild-type plants and also those hemizygous for the insertion, but not homozygous plants. Lane M shows no amplification, indicating this plant is homozygous for the insertion. An allelism test was conducted by crossing a seed parent homozygous for the mnt-1 mutation with the Salk 108995 homozygote as pollen parent. F1 progeny were hemizygous for the insertion (FIG. 5E) and had the mnt-1 mutant phenotype (FIG. 5F), confirming that MNT is the ARF2 gene.

**VIII.** Please replace the paragraph located on page 45, lines 1-19, with the following replacement paragraph:

Wild-type plants transformed with the 35S::MNT cassette described in Example 8a, b have the mnt mutant phenotype, including closed flowers for most of the plant's life cycle (FIG. 17B, top), and large seeds. Seeds from three independently transformed lines, along with wild-type plants grown under the same conditions, are shown in FIG. 17B, middle. The overall mean weight for these three lines was 25.5  $\mu$ g, compared with 15.0  $\mu$ g for the wild-type control. Expression of MNT/ARF2 was assayed in transformed and wild-type plants by semiquantitative RT-PCR (FIG. 17B, bottom) using multiplex RT-PCR with primers RTARF2-F (5'-GAGTGGGTGGAGTGTGTTG-3') (SEQ ID NO: 63) and RTARF2-R (5'-AGTTGGTTTCGTTGAGCAT-3') (SEQ ID NO: 64), and control primers to the GAPC gene, GAPC-F (5'-CACTTGAAGGGTGGTGCCAAG-3') (SEQ ID NO: 65) and GAPC-R (5'-CCTGTTGTCGCCAACGAAGTC-3') (SEQ ID NO: 66). PCR was initiated with RTARF2 primers and run for 4 cycles at an annealing temperature of 55°C, extension time 2 min. GAPC primers were added to each reaction mix and the reaction was run for an additional 22 cycles.

This showed that plants transformed with the 35S::MNT cassette did not have lower levels of MNT expression than wild-type plants; therefore the mutant phenotype was not due to cosuppression. Therefore constitutive expression of the MNT gene (such as achieved under control of the 35S promoter) provides a further method for producing large seeds.